Degradation of oxadiazon in Kalyani alluvial soil

Subhasish K Chakraborty, Anjan Bhattacharya and Ashim Chowdhury*

Pesticide Residue Laboratory, Department of Agricultural Chemistry and Soil Science, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia-741252, West Bengal, India

Abstract: Residual fate and behaviour of the herbicide oxadiazon in Kalyani soil, paddy straw and grain were studied under subtropical conditions, in West Bengal following application @ 1 kg and 2 kg ha^{-1}. Dissipation of oxadiazon in soil followed first-order kinetics and DT_{50} values ranged from 44 to 45 days. Residues at harvest in paddy grains and straw were also studied. Degradation of oxadiazon after 60 days of incubation at 28(± 1) °C in alluvial soil at water holding capacity yielded 10 metabolites of which four were characterised by spectroscopy.

Keywords: oxadiazon; soil; degradation

1 INTRODUCTION

The adoption of good agricultural practices should lead to food products free from undesirable pesticide residues. Pesticides undergo decomposition/degradation by physical and chemical as well as biochemical processes and the degraded products so formed may or may not be harmful to the environment.[1] The fate and behaviour of pesticides under biotic and abiotic conditions in different components of our ecosystem thus play an important role in the evaluation of environmental quality.

Paddy is the major cereal crop in the Indian subcontinent as well as in West Bengal. Weeds are now a serious problem in paddy as they reduce productivity. Herbicides are increasingly used in this sub-continent. These, whether applied to plant or soil, are ultimately distributed in crops, soil, water and other components of the environment. Among the different classes of herbicide, oxadiazon, a pre-emergence soil-applied oxadiazole, is now used in India, especially in paddy fields. Since relatively little research on the persistence, movement and degradation of oxadiazon in different soil types and crops has so far been reported,[2–7] the present paper investigates the residual fate and degradation of oxadiazon in alluvial soil cropped under paddy in West Bengal.

2 MATERIALS AND METHODS

2.1 Chemicals

Oxadiazon was purified by recrystallisation from benzene and then from ethanol (99.2% purity by GLC, mp 89–90 °C). An oxadiazon 250 g litre^{-1} EC (Ronstar® 25EC) was purchased from the local market. All organic solvents were analytical grade and freshly distilled prior to use, all inorganic chemicals were laboratory grade.

2.2 Analysis

2.2.1 Gas liquid chromatography

A Hewlett Packard (HP USA) Model 5890 A gas liquid chromatograph (GLC) equipped with a Ni^{63} electron-capture detector (ECD) was used to estimate oxadiazon. The column was glass (1.83 m × 2 mm ID) packed with 5% DC-200 on Chromosorb WHP (80–100 mesh). The injector, column and detector temperatures were 160 °, 210 ° and 260 °C respectively, using nitrogen as carrier gas with a flow rate of 45 ml min^{-1}.

2.2.2 GC-MS

GC-MS was used for structural assignment of oxadiazon metabolites. A Hewlett Packard Model 39928 GC-MS instrument equipped with a Machery-Nagel capillary (Parmabond SE-52; 25 m × 0.35 mm ID) coated with 0.5 μm film of phenyl methyl silicone, at an ionisation potential 70 eV was used to obtain the mass spectra. Chromatographic separation was performed using temperature programming 100–250 °C, 4 °C min^{-1} with initial temperature at 100 °C for 4 min and the carrier gas, helium, at 2 ml min^{-1}.

2.3 Residue analysis under field conditions

The experiment was conducted in paddy (variety IR-36) at the University Research Farm (alluvial soil), Bidhan Chandra Krishi Viswavidyalaya Kalyani, West Bengal, India (properties of the soil are in Table 1), during *Kharif* season (July–September, 1990). This site had no previous history of oxadiazon application.

* Correspondence to: Ashim Chowdhury, Pesticide Residue Laboratory, Department of Agricultural Chemistry and Soil Science, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia-741252, West Bengal, India
Contract/grant sponsor: CSIR New Delhi
(*Received 4 November 1998; revised version received 26 March 1999; accepted 23 May 1999*)

Table 1. Physicochemical properties of the soil

H (in water)	7.2
Organic matter (%)	1.32
CEC (meq/100 g)	13.23
Water content (air dry) (%)	3.4
Water content (saturated) (%)	45.3
Sand (0.50–0.05 mm) (%)	46.37
Silt (0.05–0.002 mm) (%)	30.15
Clay (<0.002 mm) (%)	23.48

2.3.1 Field treatment

The commercial 250 g litre^{-1} oxadiazon EC was applied seven days after transplanting (2 August 1990) to the main experiment field (plot size 8 m × 4 m each) at two rates 1.0 and 2.0 kg AI ha^{-1}. The field experiment was of a randomised block design with three replications of each treatment along with an untreated control. The plant-to-plant and row-to-row distances were 15 cm and 20 cm respectively. The formulation was diluted with water and sprayed at 500 litre ha^{-1} with a hand atomiser.

2.3.2 Sampling and extraction

The soil samples from each plot were collected by soil auger from six field locations at an intervals of 0 (1 h after application), 20, 30, 45, 70 and 90 days after herbicide application. The samples were then air-dried at room temperature, ground and mixed thoroughly. A representative air-dried sub-samples (50 g) from each replicate was taken in a 250-ml conical flask with methanol (100 ml), and after sonication for 1 h, kept overnight and then filtered through Whatman No 1 filter paper on a Buchner funnel under vacuum. The residual soil in the conical flask was again sonicated for 10 min with methanol (2 × 50 ml) and filtered as before, ready for clean-up and analysis. Straw and grain samples were taken separately from each replication at six different locations at harvesting (ie 90 days after herbicide application). Straw samples were chopped into small pieces (0.5 to 1.0 cm), mixed thoroughly and a composite straw sample (50 g) was taken for extraction. The grain sample after dehusking was powdered in a grinder and mixed thoroughly, from which a representative grain sample (50 g) was taken. The samples of straw and grain were extracted with methanol (200 ml) in a Soxhlet apparatus for 6 h and the methanol extract was then filtered through a Whatman No 1 filter paper under vacuum.

2.3.3 Clean-up and analysis

The combined methanol extracts for each sample were concentrated using a rotary vacuum evaporator at 50 °C after adding two or three drops of ethylene glycol as a keeper. The concentrated methanol extract was then transferred completely to a separating funnel and treated with saturated aqueous sodium chloride solution (150 ml), which was extracted with benzene (3 × 7.5 ml). The benzene layers were combined, passed through anhydrous sodium sulfate and concentrated (2 ml). The concentrated benzene extract was then subjected to column chromatography on a dry Florisil column, (Merck, Germany, 60–100 mesh, 8 g, 20 cm × 1.5 cm ID). The first 50 ml of hexane eluate was discarded and then 150 ml of hexane + acetone (9 + 1 by volume) eluate was collected, reduced to dryness using a rotary evaporator and the volume of the residual sample was adjusted with distilled hexane (5–25 ml) for the estimation of oxadiazon residue by GLC.

2.4. Degradation of oxadiazon in Kalyani soil

2.4.1 Collection of soil sample

To study the nature of metabolites produced from oxadiazon, a soil sample was collected from the paddy field of the University Research Farm.

2.4.2 Laboratory study

Finely sieved (80 mesh) air-dried soil (1 kg) was placed in an amber glass bottle and oxadiazon (1 mg in 1 ml acetone) was applied at the rate of 100 mg kg^{-1} based on the air-dried soil weight. The bottle was then shaken vigorously for thorough mixing of the oxadiazon and left overnight in a desiccator for complete evaporation of acetone. Sterilised distilled water then added to the soil on the basis of the water-holding capacity of the soil. The bottle was stoppered with a non-absorbent cotton wool plug and incubated at 28 (± 1) °C for 60 days. Under the laboratory conditions, approximately 3% of the water was evaporated within 48 h, so the water content was maintained by adding the calculated amount of water every three days. A control was set up with no oxadiazon application.

2.4.3 Isolation and identification of metabolites

After 60 days' incubation, soil was extracted with methanol + water (80 + 20 by volume; 1 litre) in a Soxhlet apparatus for 20 h. The extract was then filtered through a Buchner funnel fitted with a Whatman No 1 filter paper under vacuum and the filtrate was evaporated to dryness by rotary vacuum evaporator at 50 °C. The concentrated extract was then partitioned with 3 × 250 ml hexane in a separating funnel after addition of saturated aqueous sodium chloride (500 ml) solution to remove any lipids and other non-polar interfering co-extractives. The remaining aqueous layer was again extracted with dichloromethane + ethyl acetate (1 + 1 by volume; 3 × 350 ml) The hexane phase was further extracted with acetonitrile (3 × 100 ml). Both acetonitrile and dichloromethane-ethyl acetate extracts were combined, passed through anhydrous sodium sulfate and concentrated to dryness under reduced pressure at 50 °C and redissolved in dichloromethane (500 ml). This dichloromethane extract was then transferred to a 1-litre separating funnel and washed with sulfuric acid (0.5 M; 3 × 100 ml). The acidic phase was further extracted with dichloromethane (2 × 100 ml) and the combined dichloromethane extracts washed with

Table 2. Summary of method validation data, recovery of oxadiazon from soil, paddy straw and grain

Substrate	Amount fortified ($\mu g\ g^{-1}$)	Recovery of oxadiazon (%)	
		Range	Mean ($\pm$ SD)[a]
Soil	1.00	94.2–99.4	96.3 ($\pm$2.6)
Paddy straw	1.00	83.4–86.5	84.6 ($\pm$1.7)
Paddy grain	1.00	84.9–89.7	89.7 ($\pm$3.2)

[a] Four replicates.

aqueous sodium bicarbonate (50 g litre^{-1}; 2×100 ml) solution. Water and sodium bicarbonate phases were re-extracted with dichloromethane (2×100 ml). Dichloromethane extracts were combined, dried over anhydrous sodium sulfate, concentrated under reduced pressure in a rotary vacuum evaporator and then chromatographed over a Florisil column (1 cm ID $\times$ 40 cm long) packed and pre-washed with hexane. Concentrated dichloromethane extract was poured onto the head of the column for adsorption by Florisil and a small quantity of anhydrous sodium sulfate was laid on the top of the column. Elution was carried out successively with 100 ml each of hexane, hexane + acetone (95 + 5; 9 + 1; 8 + 2 and 1 + 1 v/v) and finally with acetone (100%) and the eluates were designated as column fractions 1 to 6 respectively. Then these fractions were subjected to GC-MS analysis for identification and characterisation of oxadiazon and its metabolites.

3 RESULTS AND DISCUSSION

3.1 Efficiency of analytical method

Recoveries of oxadiazon from the different substrates varied from 84 to 96% (Table 2) being greatest from paddy soil (96.3%) and least from straw (84.6%). Control samples showed little interference (detectable limit 0.005 $\mu g\ g^{-1}$).

3.2 Persistence of oxadiazon under field conditions in soil, straw and grain

Initial deposits of oxadiazon in soil immediately after application (0 days) were 2.4 ($\pm$0.11) $\mu g\ g^{-1}$ and 5.2 ($\pm$0.08) $\mu g\ g^{-1}$ for lower and higher rates respectively. After 20 days the parent residues declined sharply between 20 and 30 days, with losses of 37.5% and 43.0% in the lower and higher treatment doses by 30 days.

Meterological data over the period of this study (Table 3) indicated typical temperatures (average maxium 35.2 °C and minimum 10.4 °C); sunshine hours were low to moderate and rainfall frequent. Heavy precipitation was recorded during the period of 0–20 days (92.4 mm), 30–45 days (100.2 mm) and 45–70 days (221.1 mm) after herbicide application but, during these periods, losses of oxadiazon from paddy soil were low. Again maximum loss was observed during the period, the cumulative loss was not so high. This fact can also be substantiated from the earlier study by Carringer *et al*,[8] who suggested that the mechanism of adsorption of oxadiazon by organic matter and clay colloid was hydrophobic in nature. Besides this, Paulo *et al*[9] reported that oxadiazon leached 3 cm in the soil column under simulated rainfall of 60 to 120 mm. This observation was also well in accordance with that reported by Ambrosi and Helling.[10] Therefore, loss of oxadiazon through leaching due to heavy rainfall was minimal.

As the vapour pressure of the herbicide is low, volatilisation should not be a path for loss of oxadiazon from soil.[11,12] Weather influences temperature and moisture in soil, which in turn affects degradation rates. The major pathways of potential degradation are microbial, chemical and photochemical. Kawamuro *et al*[13] reported that soil organic matter content played a vital role for the dissipation of oxadiazon under flooded conditions, but soil pH, clay content, CEC and bulk density, all had effects. Recently Li and Wang[5] observed that oxadiazon was dissipated very quickly in soil of high clay content and that microorganisms played a minor role in this degradation. Similarly Finkel'shtein *et al*[14] were not able to isolate any microbial strain which could effectively degrade oxadiazon. Therefore, it can be assumed that plant uptake and soil characteristic such as soil pH, CEC, texture, organic matter content, etc are the main factors responsible for loss of oxadiazon from soil.

3.3 Terminal/harvested residues of oxadiazon in paddy straw and grain

Paddy straw at 90 days after application had residues

Table 3. Meteorological data during the study of oxadiazon dissipation in Kalyani soil

Days between sampling	Temperature (°C)		Average relative humidity (%)		Cumulative rainfall (mm)	Cumulative sunshine (h)
	Min	Max	Min	Max		
0	33.5	25.5	90.0	80.0	0.4	0.00
0–20	33.8	24.0	91.5	78.70	92.4	5.02
20–30	34.2	24.9	92.1	73.50	42.7	6.28
30–45	35.2	23.9	95.2	83.10	100.2	5.24
45–70	33.4	21.2	94.2	81.48	221.2	6.25
70–90	32.2	16.4	94.6	57.90	7.7	7.12

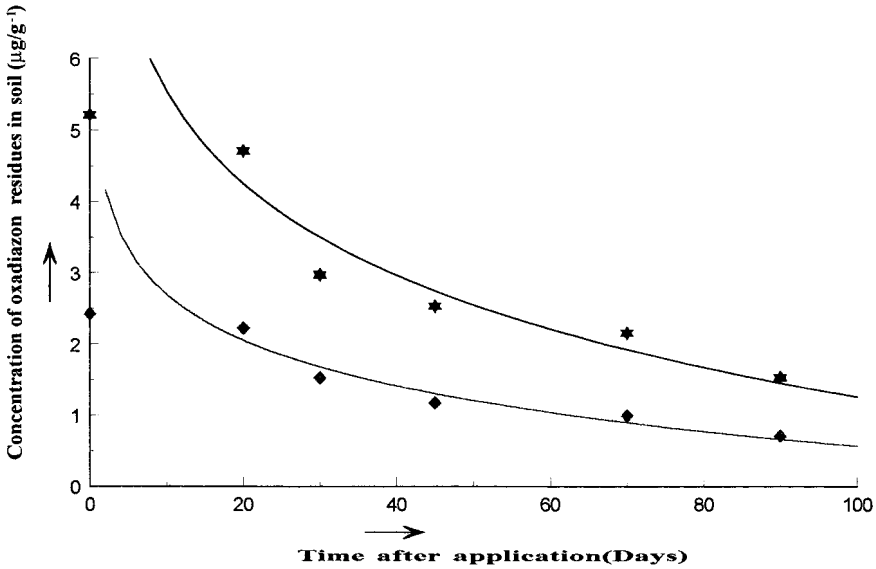

Figure 1. Rate of degradation of oxadiazon in alluvial soil under paddy cultivation. (◆) 1.0 kg AI ha^{-1}, (*) 2.0 kg AI ha^{-1}.

of 0.16 (±0.07) and 0.61 (±0.11) μg g^{-1} at 1.00 and 2.00 kg AI ha^{-1} whereas 0.016 (±0.006) and 0.041 (±0.014) μg g^{-1} were detected in grain samples. Therefore, oxadiazon was taken up by the roots of paddy and subsequently distributed to shoots and grain, but the maximum accumulation was observed in the straw. It was observed by Ishizuka *et al*[2] that oxadiazon translocated from roots to shoots and was distributed more in the aged leaves than into younger leaves. They also found that concentration of oxadiazon decreased in the plant parts in the order leaves + stem ≫ husks ≫ hulled grains.

Residues of oxadiazon in soil plotted against time after application (Fig 1) showing the rate of degradation of oxadiazon in alluvial soil declined gradually with time at both rates of applications. The $T_{1/2}$ under field condition at 1.0 and 2.0 kg AI ha^{-1} was found to be 44 and 45 days respectively. Thus, it has been shown that oxadiazon is persistant in paddy soil, as similar findings have also been reported by other workers. Ambrosi *et al*[11] observed that less than 25% oxadiazon was degraded after 175 days in soil under both moist (75% field capacity) and flooded conditions. In another study Li & Wang[5] found 73 to 96% of the applied herbicide remaining after 144 days in three different soils that were saturated with water or flooded. Rhone-Poulenc[15] report a half life of two to six months for oxadiazon in soil.

3.3 Degradation of oxadiazon in Kalyani soil

3.3.1 Isolation and characterization of the metabolities of oxadiazon from Kalyani soil

GC-MS of six different column fractions indicated the formation of 10 metabolites of oxadiazon in soil. The mass fragmentation patterns of these, including parent oxadiazon, are presented in Tables 4 and 5. Of these 10 metabolites, the structure of four (**II**–**V**) were confirmed by spectroscopy. The MS data for all other six metabolites (unknown **1** to unknown **6**) were not sufficient for elucidation of their structures. The mass spectral data of metabolites **II** and **IV** are in agreement with those reported elsewhere.[15] The structure of

Table 4. Mass fragmentation of identified metabolites and parent oxadiazon

Product isolated	*Column fraction*	*GC-MS R_1 (min)*	*M^+ (m/z; relative abundance%)*	*Other major fragments (m/z; relative abundance, %)*
I (Oxadiazon)	2	30.7	344 (16.8)	348 [(M^++4), 2.3]; 346 [(M^++2), 4.9]; 302 [($M^+ - C_3H_7$+H), 12.5]; 258 [(302 − CO_2)$^+$, 25.5]; 202 [(258 − C_4H_6+H); 12.3], 175 [(202 − HCN)100].
II	1	12.20	204 (11.4)	208 [(M^++4), 2.8]; 206 [(M^++2), 6.2]; 162 [$M^+ - C_3H_7$+H), 100]; 133 [($M^+ - C_3H_7$ − CO); 14.6]; 126 [($M^+ - C_3H_7$ − Cl), 7.8]; 98 [(133 − Cl).26.1]
III	2	21.42	219 (11.4)	223 [(M^++4), 1.6]; 221 [(M^++2), 4.8]; 177 [$M^+ - C_3H_7$+H), 18.7]; 149 [(177 − CO)$^+$, 100]; 121[($M^+ - C_3H_7$ − CO-HCN), 5.9].
IV	3	32.06	318 (12.0)	322 [(M^++4), 1.7]; 320 [(M^++2), 4.3]; 276 [($M^+ - C_3H_7$+H), 12.9],241 [(276 − Cl), 12.0]; 235 [($M^+ - COC(CH_3)_3$+2H), 100]; 200 [(276 − C_4H_9), 12.0]; 192 [(276 − $COC(CH_3)_3$+H), 15.1]; 176 [(276 − $COC(CH_3)_3$ − NH),19.2]; 149 [($M^+ - C_4H_9 - COC(CH_3)_3$ − NH − HCN),12.2]; 85 [(−$COC(CH_3)_3$]$^+$ − 69.3].
V	3	27.11	316 (18.2)	320 [(M^++4), 2.1]; 318 [M^++2), 8.5]; 272 [$M^+ - CO_2$), 13.6]; 189 [272 − C_4H_9 − HCN-H), 100], 174 [(189 − CH_3), 11.3]; 149 (24.3].

Table 5. Mass fragmentation of unidentified metabolites

Product isolated	*Column fraction*	*GC-MS R_t min*	*Major ion fragments (m/z; relative abundance%)*
Unknown **1**	3	29.52	310 (M^+ 11.9) 253 [$M^+ - C_4H_9$), 12.2]; 224 [($M^+ - C_3H_7 - CO_2 + H$), 12.6]; 168 [$224 - C_4H_9 + H$), 12.5]; 141 [(168 – HCN), 20.2]; 57 [($-C_4H_9$), 100].
Unknown **2**	4	31.7	294 (M^+ 11.6) 263 [($M^+ - OCH_3$), 235 [($M^+ - COOCH_3$), 15.7]; 209 [($M^+ - COC(CH_3)_3$, 6.6];178 [(235 – C_4H_9), 5.7]; 150, [(209 – $COOCH_3$), 10.4], 135 [(150 – NH), 12.8]; 109 [11.3];91 [12.3], 81 (100).
Unknown **3**	4	31.2	280 (M^+ 12.4) 249 [($M^+ - OCH_3$), 13.9]; 220 [($M^+ - COOCH_3 + H$), 39.4]; 165 [(220 – C_4H_9),18.9]; 136 [($M^+ - COOCH_3 - COC(CH_3)_3$, 13.4]; 121 [(136 – NH), 15]; 116 [(121 – CH_3),100]; 94 [(116 – HCN), 32.3].
Unknown **4**	4	33.78	340 ($M^+ - C_3H_7 - CH_2 + CH)^+$, 16.7%], 241 [(284 – CO_2 + H), 100%], 185 [(241 – C_4H_9 + H),44.4], 171 [($M^+ - C_3H_7 - C_4H_2 - CO_2 - HCN + 2H$)].
Unknown **5**	5	25.3	278 (M^+ 10.1) 224 [($M^+ - C_4H_9 + H$, 26.6]; 151 [($M^+ - COCH_3 - COC(CH_3)_3 + H$), 100]; 123 [15 – C_2H_5 + H), 11.9], 95 [(123 – CO), 13.2].
Unknown **6**	6	28.18	266 (M^+ (13.0) 209 [($M^+ - C_4H_9$), 8.2]; 207 [($M^+ - COOCH_3$) 11.1]; 183 [($M^+ - COC(CH_3)_3$,11.83]; 179 (14.5), 123 [(207 – $COC(CH_3)_3$, 11.33, 87 [(M^+ – 179, 100].

metabolite **IV** was also confirmed by IR and [^{1}H]NMR of the authentic compound.[16] Three metabolites (**II, III** and **IV**) are reported for the first time as products of oxadiazon in soil. The metabolite **V** has also been reported as one of metabolites of oxadiazon in soil,[11] rice plant,[2] snails[4] and green and dry hops and hop foliage.[7]

Based on the metabolites of oxadiazon isolated and characterised so far from Kalyani soil, a scheme representing a plausible pathway of oxadiazon degradation is presented (Fig 2). The main degradative pathways of oxadiazon in soil were C-dealkylation, heterocyclic ring cleavage and *N*- decarboxylation. The metabolite V was formed by C-dealkylation, whereas heterocyclic ring cleavage of oxadiazon yielded an intermediate **VI** which underwent *N*- dealkylation to give compound **IV**. Complete elimination of the oxadiazoline ring from the aromatic ring system formed metabolite **II**. Besides these, out of four metabolites of oxadiazon produced in soil, two metabolites (**II** and **IV**) are also formed as the degradative products of oxadiazon during co-metabolism by the soil-borne fungus *Fusarium solani* (Mart) Sacc[16] Therefore, these two metabolites might be formed in soil via microbial processes.

ACKNOWLEDGEMENTS

The authors are thankful to M/s Rhone-Poulenc (India) Ltd for supplying technical grade oxadiazon and one of the authors (SKC) gratefully acknowledge the financial support from CSIR New Delhi (India) as Senior Fellowship for carrying out this work.

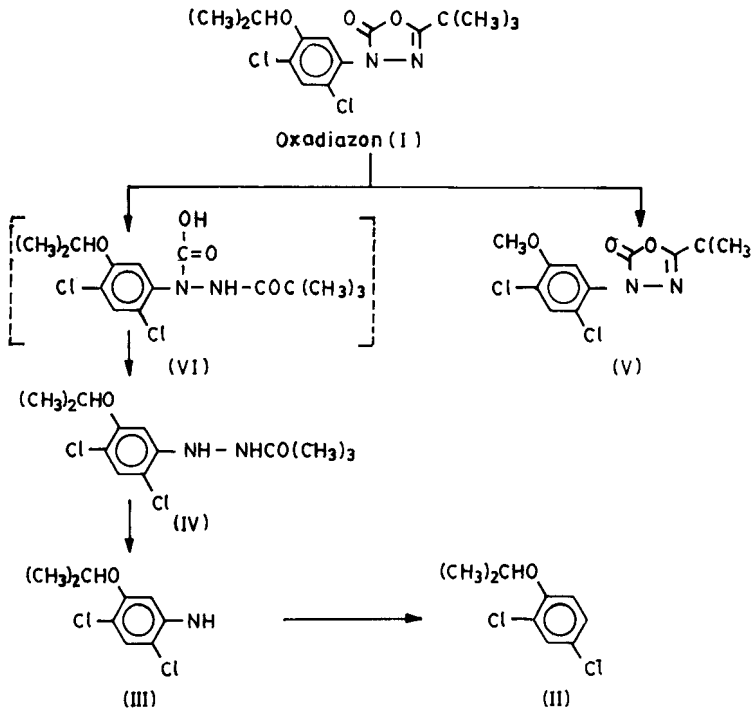

Figure 2. Postulated pathways of oxadiazon degradation in Kalyani soil.

REFERENCES

1 Wallnofer PR and Engelhardt G, Microbial degradation of pesticides; in *Degradation of Pesticides, Desiccation and Defoliation, AcH Receptors as Targets*, ed by Richter R, Otto D and Menges HJ, Springer-Verlag, New York 1–115 (1989).

2 Ishizuka K, Hirata H and Fukunaga K, *Agr Biol Chem* **39**:1431–1446 (1975).

3 Hirata H and Ishizuka K, *Agr Biol Chem* **39**:1447–1454 (1975).

4 Ambrosi D, Isensee AR and Macchia JA, *J Agric Food Chem* **26**:50–53 (1978).

5 Li GC and Wang SS, *Weed Sci Bul Taiwan* **1**:52–59 (1980).

6 Mengoes RM and Tamez S, *Weed Sci* **29**:74–79 (1981).

7 George DA, *J Assoc Off Anal Chem* **65**:28–31 (1982).

8 Carringer RD, Weber JB and Monaco YJ, *J Agric Food Chem* **23**:568–572 (1975).

9 Paulo FM, Signori LH and Deuber R, *Planta Daninha* **2**:111–115 (1975).

10 Ambrosi D and Helling CS *J Agric Food Chem* **25**:215–217 (1977).

11 Ambrosi D, Keaney PC and Macchia JA, *J Agric Food Chem* **25**:868–872 (1977).

12 Barrett MR and Lavy TL, *Weed Sci* **32**:697–701 (1984).

13 Kawamura Y and Hirai K, *Proc 5th Asian Pacific Weed Sci Soc Conf*, Tokyo, Japan. 155 (1976).

14 Finkel'shtien ZI, Nefedova MY, Baskunov BP, Utepbergenova KS and Golovleva LA, *Agrokhimiya* **4**:88–91 (1984).

15 Anon, Rhone-Poulenc, *Ronstar Herbicide* Rhone-Poulenc Agro-Chemicals, Paris, France 19 (1978).

16 Chakraborty SK, Chowdhury A, Bhattacharyya A, Ghosh S, Pan S, Waters R, and Adityachaudhury N, *J Agric Food Chem* **43**:2964–2969 (1995).